SIGNAL COUNTING FOR IN SITU HYBRIDIZATION

ABSTRACT

Fluorescently tagged nucleic acid probe signals are counted in biological specimens by determining a ratio of signals from a test probe to signals of a reference probe. Probe signals need not be counted with reference to cells, nuclei, or nuclear contours. Gene amplification or deletion can thus be detected by analyzing the ratio. Successive image slices are obtained by confocal microscopy, and the images are digitized. The digital images are transformed and analyzed to combine contiguous fluorescent signal segments in successive optical sections to identify discrete probe signals, or spots. Spots overlapping in the axial and transverse dimensions of a three-dimensional representation of the biological specimens can be distinguished. A graphical user interface presents various features for consideration by a user, who can provide guidance to a computer system counting the spots. Various features directed to identifying spot clusters and autofluorescent material can increase accuracy of spot counting.